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PATENT & TRADEMARK OFFICE

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE		
	Application No.	10/060,369
	Filing Date	February 1, 2002
	First Named Inventor	Roman ZASTAWNY
	Group Art Unit	1646
	Examiner Name	Not Yet Assigned
	Attorney Docket No.	2931-104
Title of the Invention:	**G PROTEIN COUPLED RECEPTOR A4**	

RESPONSE TO NOTICE FILE CORRECTED APPLICATION PAPERS AND PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In response to the Notice to File Corrected Application Papers and prior to examination on the merits, please enter the amendments contained on the following pages, which are summarized below.

IN THE SPECIFICATION:

Substitute the following paragraphs in the specification as follows.

Marked-up copies of the original text of the specification pages are attached to this amendment. Material inserted is indicated by redlining (redlining) and material deleted is indicated by strikeout (~~strikeout~~),

04/02/2003 MBLANCO 00000012 10060369
01 FC:2253 465.00 OP

Clean Copy of Substitute Specification Page 3, second, fourth, fifth, and sixth paragraphs:

Figure 1 provides a polynucleotide encoding the human A4 receptor (SEQ ID NO: 8) and the predicted amno acid sequence (SEQ ID NO: 9).

Figure 3 provides the sequence alignment between the predicted amino acid sequnce of the human A4 receptor (SEQ ID NO: 9) and the human Y1 receptor (SEQ ID NO: 10).

Figure 4 provides the sequence alignment between the predicted amino acid sequence of the human A4 receptor (SEQ ID NO: 9) and the human Orexin Receptor -2 (SEQ ID NO: 11).

Figure 5 provides the sequence alignment between the predicted amino acid squence of the human A4 receptor (SEQ ID NO: 9) and the human CCK receptor (SEQ ID NO: 12).

Clean Copy of Substitute Specification Page 16, second full paragraph: Showing changes made

To identify sequences corresponding to the 5' -end of AA449919 open reading frame a 5' -RACE PCR technique was utilized. Two 5' directed primers, sequence P1 (5'GAGACATAAT-GGTGATGGCTAGGACCCA 3') (SEQ ID NO: 1) and P2 (5' CTGCGACAGATATTCCCT-GGACCAATCC 3') (SEQ ID NO: 2) were designed based on the sequence of the AA449919 cDNA clone. These oligonucleotide primers were used in a 5' RACE PCR procedure to obtain the upstream sequences from human brain Marathon-Ready ™ cDNA Amplification Kit (Clontech Laboratories Inc.; Cat. No. 7400-1) according to the manufacturers recommendation. Human brain cDNA was amplified using primer P1 and the adaptor primer AP1, (5' CCATCCTAATACGACTCACTATAGGC 3'; Clontech) (SEQ ID NO: 3) under the following PCR conditions: 1 min at 94 °C; 5 cycles of 30 seconds at 94°C then 4 minutes at 72°C; 5 cycles of 30 seconds at 94°C then 4 minutes at 70°C; 25 cycles of 30 seconds at 94°C then 4 minutes at 68°C; 10 minutes at 68°C; 10 minutes at 68°C. An aliquot of this PCR reaction was diluted and re-amplified under the same cycling conditions using the primer AP2 (5'

ACTCACTATAGGGCTCGAGCGGC 3') (SEQ ID NO: 4) which is nested with respect to AP1, and primer P2, which is nested with respect to P1. An aliquot of this reaction was electrophoresed on a 1% agarose gel. A band of 600 bp was visible by ethidium bromide staining. Eluate of this band was re-amplified with primers AP2 and P2 under the following PCR conditions: 1 min at 94°C, 30 cycles of 30 seconds at 94°C, 30 seconds at 70°C, 1 minute at 72°C, 10 minutes at 72°C. An aliquot of this PCR reaction was run on a gel and ethidium bromide staining revealed a band at the expected size of 600 bp. An aliquot of the PCR reaction was used directly for ligation to the vector pCR 2.1 (Invitrogen, Cat. No. K2030) and transformed into Top 10F' bacterial cell. The resulting clones were sequences by the dideoxy chain termination method on an Applied Biosystems Model 377 fluorescent dye DNA sequencer. This 600 bp clone overlapped the AA449919 cDNA sequence and included sequences representing the entire 5' end of the open reading frame including the codon representing the entire 5' end of the open reading frame including the codon representing the initiating methionine as well as some 5' UTR sequences.

Clean Copy of Substitute Specification Page 17, second paragraph: Showing changes made

EXAMPLE 2

Reconstruction of a full-length human A4 clone using PCR

The DNA sequence encoding for the novel receptor A4 was amplified using oligonucleotide primers corresponding to the 5' and 3' end of the cDNA. The 5' oligonucleotide primer, termed PA4-5, has the sequence 5' -GGCATTCGAATTCGCCGCCACCATG-AATGAGAAATGGGACACAAACTCTT-3' (SEQ ID NO: 5) and contains a EcoRI restriction site and a consensus Kozak translation initiation sequence follows by 28 nucleotides of the AA449919 sequence starting from the methionine start codon. The 3' olglionucleotide primer termed PA4-3, has the sequence site (5' -AGGATTATCACTCTAGATCTTTTTAAATCT-CACTGCTGTTAGTAGTTTCT-3' (SEQ ID NO: 6) and contains 33 bases of the 3' UTR of the AA449919 sequence and a Xbal restriction site. A full-length sequence encoding for the A4

receptor was obtained using a two step procedure. First, two cDNA clones corresponding to the 5' and 3' ends of the AA449919 open reading frame were amplified in separate reactions. Second, the two products were combined and reamplified in the presence of human kidney Marathon-Ready ™ cDNA with appropriate primers to give a full-length A4 cDNA clone. Briefly, amplification of the 5' fragment was performed using the human kidney Marathon-Ready ™ cDNA Amplification Kit (Clontech Laboratories Inc.; Cat. No. 7405-1) with primers PA4-5 and P1 under the following PCR conditions: 1 min at 94°C; 25 cycles of 30 seconds at 94°C, 4 minutes at 72°C; 10 minutes at 72°C. An aliquot of this reaction was reamplified under the same conditions and produced a band of the proper size when an aliquot of the reaction was run on an ethidium bromide stained agarose gel. Amplification of the 3' fragment was also performed using human kidney cDNA by amplification with primer P5 (5' -TGGCACGTGGTGTCC-AGGAA-GAAGCAG-3) (SEQ ID NO: 7) and PA4-3 under the following PCR conditions: 1 min at 94°C; 35 cycles of 30 seconds at 94°C, 30 seconds at 65°, 4 minutes at 72°C; 10 minutes at 72°C. A strong band of the proper size was visible when an aliquot of the reaction was run on an ethidium bromide stained agarose gel. Next, to produce a full-length cDNA human kidney marathan cDNA was combined with two above PCR products and extended using PA4-5 and PA4-3 primers under the following conditions: 1 min at 94°C; 35 cycles of 30 seconds at 94°C, 30 seconds at 65°C, 4 minutes at 72°C; 10 minutes at 72°C. An aliquot of the first round of PCR was then re-amplified under identical conditions except the cycle number was increased to 35. A strong band and of the proper size (1.4 kilobases) was visible when an aliquot of the reaction was run on an ethidium bromide stained agarose gel. An aliquot of the PCR reaction was restriction digested with the enzymes EcoRI and Hind III and electrophoresed on an 1% agarose gel. The PCR product was excised, purified, ligated into the EcoRI/Xbal sites of the mammalian expression vector pcDNA3 (Invitrogen). The resulting construct, named pcDNA3-A4. Orientation of the cDNA was confirmed by restriction digestion analysis and sequencing.

REMARKS

In response to a notification to file corrected application papers dated October 29, 2002 (a response copy is attached), an initial Sequence Listing is submitted, and its entry into the application is respectfully requested. An initial computer-readable form of the Sequence Listing is also submitted, and it is hereby certified that the content of the Sequence Listing information recorded in the computer readable form is identical to the Sequence Listing written on paper and contains no new matter. The amendments to the specification have been made to properly include the sequence identifiers or to correct obvious typographical errors.

RESPECTFULLY SUBMITTED,					
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Attachments: Marked-Up Copies of Specification Amendments

2931-104.preliminary.wpd

Amended Specification Page 3, second, fourth, fifth, and sixth paragraphs:: Version with markings to show changes made

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Amended Specification Page 16, second full paragraph: Showing changes made

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